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Penicillins as pharmaceuticals for the downregulation of IFNγ production

Specification

The present invention relates to the use of penicillins as therapeutic agents for the treatment of autoimmune diseases or other pathological situations where IFNγ-mediated effects are involved in the progression of the disease and to hapten-modified peptides for use as pharmaceutical compounds and/or diagnostic tools for the determination of a predisposition for hypersensitivity reactions and for the desensitization of patients suffering from hypersensitivity reactions against penicillins or parts or derivatives thereof.

T lymphocytes comprise a central part of the immune system in vertebrates. Their differentiation upon antigenic stimulation plays a critical role in adverse immune responses including autoimmunity and hypersensitivity reactions.

Specific T cell activation is achieved exclusively by interaction of specialized antigen receptors (TCR), clonally distributed on T lymphocytes with presenting molecules, encoded by the major histocompatibility complex (MHC) genes on competent cells, associated to the antigenic stimuli (peptidic antigen). Cytotoxic T cells (Tc) require class I MHC molecules, while helper T cells (Th) need class II MHC molecules. This so-called MHC restricted antigen recognition results from stringent selection processes during T cell maturation in the thymus.

Upon initial exposure to a foreign antigen, T cells can differentiate into type 1 or type 2 phenotype, functionally different. Type 1 cells secrete mainly IFNγ and are involved in cell-mediated adverse reactions. Type 2 cells produce IL4 and drive antibody-mediated immune responses (Ref. 8). In

cases where polarized responses are dominant, the antigen dose appears as one of the critical parameters in determining naive T cell differentiation (Ref. 9). For peptidic antigens, it has been shown that high antigen doses drive IFNγ production and type 1 differentiation of naive T cells, while low doses of the same antigen lead to IL4 secretion and type 2 differentiation.

Using as model the penicillin antibiotics, the inventors examined the modulation of T cell cytokine pattern upon hapten stimulation.

A principal object of the present invention was to provide possibilities to influence the T cell cytokine pattern and to provide pharmaceuticals which can be used to treat patients suffering from diseases which are caused by overreactions or malfunctions of the immune system.

This object is solved according to the invention by the use of penicillins for the treatment of autoimmune diseases or other pathological situations where IFNγ mediated effects are involved in the progression of the disease. In another embodiment of the invention the object is solved by the use of one or more penicillin for the production of a pharmaceutical for the treatment of autoimmune diseases or other pathological situations where IFNγ mediated effects are involved in the progression of the disease.

As already shown for other antigen specific reactivities, also the specific immune response to penicillins is dose dependent, as can be seen from the examples included in this specification. In the context of the present invention, it was possible to show that penicillins can be used successfully to downmodulate IFNγ expression.

For the use according to the invention principally all ß-lactam antibiotics can be used as a penicillin except ampicillin. In preferred embodiments of the invention, Pen V and Pen G are used to block the IFNγ production of ongoing immune responses.

The finding of the invention that penicillins, and preferably Pen V and Pen G, can be used to downmodulate IFNγ production provides a new and promising possibility for therapy of autoimmune diseases or other pathologies where IFNγ mediated effects are involved in the progression of the diseases or at least improvement of the situation of the patients.

A further object ot the invention is the identification of hapten-modified MHC- binding peptides which can be used as molecular antigen inducing MHC-restricted adverse drug-specific immune reactions.

It has long been known that T cells may react to a multitude of chemical reagents, including drugs, often resulting in adverse immune reactions. It was therefore an object ot the present invention to provide compounds that allow to study the interactions of certain drugs and the components of the immune system. It was a further object of such molecules for patients to benefit of their properties. Especially it was desirable to provide diagnositc tools to identify specific T cells that are able to provide possibilities to prevent certain types of allergic reactions.

In this context the invention provides hapten-modified peptides for use for the production of pharmaceutical compounds and/or diagnostic tools, wherein the peptide contains a backbone of amino acids wherein on at least one of these amino acids a penicillin antibiotic is bound.

In preferred embodiments of the invention, the penicillin antibiotic is Pen G, Pen V or ampicillin (amp).

It was discovered (Ref. 6, 7) that hapten-modified peptides which are to be used according to the invention are especially suitable when their amino acid backbone consists of 8 to 20 amino acids, preferably 10 to 15 amino acids. In a further preferred embodiment the amino acid backbone contains at least one lysin onto which the antibiotic is bound. In still a further

preferred embodiment the hapten-modified peptide contains a tyrosine on the amino acid backbone. The tyrosine is preferably present, since it serves as anchor to the MHC molecules. Once the hapten-modified peptide is anchored to the MHC molecule, it has the right conformation to contact and eventually bind specific TCR.

In the further preferred embodiment the lysin onto which the antibiotic is bound is located in position 3, 5 or 8 calculated from the tyrosine anchor on the amino acid backbone.

The hapten-modified peptides according to the invention are useful as pharmaceutical compounds and/or diagnostic tools. One diagnostic application for the hapten modified peptides is the screening of patients for a predisposition for hypersensitivity reactions against penicillins in general. In a preferred embodiment of the invention PBMCs (peripheral blood mononuclear cells) are isolated from patients with suspected predisposition of allergic reactions. PBMCs are easily purified from the blood of patients. They are stimulated in vitro by addition of the hapten-modified peptides according to the invention and the proliferation of antigen responsive T cells is measured.

In view of the fact that such responsive T cells bind via their TCR to the hapten-modified peptides, such T cells can be detected by an immunoassay using the hapten-modified peptides as capture reagent. Immunoassay protocols for conducting such assays are known in the art and can easily be adjusted to the present object.

The invention, however, is not to be restricted to such immunoassays but every other possibility to measure proliferation of antigen responsive T cells is also encompassed by the present invention.

Since such proliferating T cells produce IFNγ and IL4 a further preferred possibility to measure proliferation lies in the monitoring of production of these substances.

Since the hapten-modified peptides according to the invention very potently stimulate T cell proliferation a further use of these substances lies in their application as pharmaceuticals for the desensitization of patient suffering from hypersensitivity reactions against penicillins or parts or derivatives thereof. This use for desensitization is a further object of the invention.

It is easily feasible to combine the peptides according to the invention with penicillin haptens, against which the allergic reaction is directed. Therefore, for patients that show hypersensitivity against for example Pen G, Pen G is used as hapten.

The hapten-modified peptides according to the invention can easily be produced in pure form. This enables a safe desensitization without the risk of further adverse reactions to the antibiotic molecule which, once covalently bound to the peptide, looses its chemical reactivity. The production of the hapten-modified peptides also is not expensive and the overall desensitization might become much cheaper as it is at present.

It is also preferred to use parts or derivatives of the penicillins for the desensitization treatment. Such parts of the molecule might be useful for the hyposensitization, but might not be as immunogenic as the complete drug molecule itself. Only parts of the penicillin molecules can therefore provide a possibility of safer and less radical treatments.

The following examples together with the figures are intended to further illustrate the invention.

Example

1. Antigens

Pen G, Pen V and Amp were obtained from Sigma, St. Louis, MO. PHA was from Murex Diagnostics Ltd., Dartford, UK. The tetanous toxoid peptide 616-630 (VRDIIDDFTNESSQK) was synthesized by continuous-flow solid-phase peptide synthesis. For all antigens, a 10-fold concentrated stock solution was freshly prepared in PBS and added to the wells at the time of experiment.

2. Culture media

The complete culture medium used in this study was RPMI 1640 supplemented with 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 1 mM sodium pyruvate and 1x mixture of non-essential amino acids (Gibco BRL, Paisley, Scotland). The medium was prepared without penicillins or any other antibiotics. For growing T cell clones the complete RPMI medium was used with addition of 5% pooled human AB serum (HS; Swiss Red Cross, Bern Switzerland) and 250 U/ml of recombinant human IL2 (Proleukin, EuroCetus, Ratingen, Germany). EBV-transformed B-lymphoblastoid cell lines were grown in complete RPMI supplemented with 10% heat-inactivated fetal calf serum.

3. Primary culture

Donors BAM, CAS and ES had a positive history of penicillin allergy and their clinical data are summarized in Table I. Primary cultures were prepared from freshly isolated PBMC in two parallel 96 well plates. Cells at a concentration of 3×10^5/w were stimulated with 3-fold dilutions of Pen G from 3 mg/ml to 0.1 μg/ml. After 5 days incubation at 37°C in 5% CO_2, the proliferative response was determined, in one of the plates, as the ^{3}H-thymidine incorporation after 16 h incubation with 1 mCi = 37 kBq ^{3}H-thymidine (Dupont, Boston, MA). Cells were harvested on GF/A-filters (Dunn Labortechnik GmbH, Asbach, Germany) and the incorporation was

measured in an automatic β-counter (Inotech, Asbach, Germany). The second plate was further expanded in IL2-containing medium for 4 days. Cells were then pooled for any given Pen G concentration, washed and rested in the absence of IL2. After another 4 days, cells were harvested and analysed for TCR expression by cell surface staining and IL4/IFNγ production by intracellular staining.

4. T cell clones

T cell clones were generated from Pen G specific T cell lines. Freshly isolated PBMC were stimulated with 1, 0.3, 0.1 mg/ml of Pen G at a density of 2 x 10^5 cells/well in a final volume of 200 μl of complete RPMI containing 5 % HS in 96-well flat bottom culture plates. After 5 days at 37°C and 5 % CO_2, 25 U/ml of IL2 was added to the wells. After another 4 days the cultures were pooled, rested for 3 days in the absence of IL2 and subjected to a second round of restimulation using irradiated autologous PBMC as APC and the same dose of Pen G given during the first restimulation. After 3 days the different cultures were again expanded in IL2 containing medium and then cloned by limiting dilution. T cell blasts were seeded at 0.3 cells/well in Terasaki plates (Nunc, Roskilde, Denmark) in the presence of 1 μg/ml PHA and 10^4 irradiated allogeneic PBMC in complete RPMI medium containing 5 % HS and 250 U/ml IL-2. The T cell clones obtained were maintained in culture by periodic stimulation in the presence of irradiated allogeneic PBMC, PHA and IL2.

5. EBV-transformed B cells

Autologous EBV-transformed B-lymphoblastoid cell lines (EBV-B cells) were prepared by culturing fresh isolated PBMC in complete RPMI containing 10 % FCS, 30 % supernatant of the EBV-producing marmoset cell line B95-8 (American Type Culture Collection, ATCC, Rockville, MD) and 600 ng/ml cyclosporin A (Sandoz, Basel, Switzerland). After overnight incubation, cells were washed and cultured in complete culture medium.

6. Flow cytometric analysis of intracellular IFNγ and IL4 production

Intracellular staining for cytokines was performed following the protocol described by Murphy et al. (12), with few modifications. Briefly, cells were resuspended at 10^6/ml in 96 well plates in a final volume of 200 µl/w of culture medium and stimulated with PMA at 50 ng/ml plus ionomycin at 500 ng/ml for 5 h at 37°C. Brefeldin A at 10 µg/ml was then added to the wells and incubation was prolonged for another 4 h. Cells were then washed and resuspended in 100 µl/w of PBS before adding an equal volume of 4 % formaldehyde fixative. After fixing for 20 min at room temperature, cells were either stored at 4°C over night or stained immediately for intracellular cytokines. For intracellular staining, all reagents and washes contained 1 % BSA and 0.5 % saponin, and all incubations were at room temperature. Cells were first washed and permeabilized for 30 min. After washing, blocking mouse IgG (Jackson Immunoresearch Laboratories, Inc., West Gove, PA) at a concentration of 300 µg/ml were given to the well for 10 min. Then, PE-conjugated anti human IL4 antibody (Clone 8D4-8, Pharmingen, San Diego, CA) plus FITC-conjugated anti human IFNγ antibody (Clone 4S.B3, Pharmingen) or isotype matched controls (PE- and FITC-conjugated mouse IgG1, Pharmingen) at a final concentration of 5 mg/ml, were directly added to the wells. After 20 min, cells were washed twice in PBS/BSA/saponin and then with PBS/BSA to allow membrane closure. Samples were resuspended in PBS/BSA and analyzed on a FACScan flow cytometer (Becton Dickinson & Co.). Thresholds were set on control stainings, included for every sample; 50,000 cells/sample were acquired. Results were analyzed using Cellquest software. T cells were detected by standard surface staining using the PE-conjugated anti human TCR$\alpha\beta$ antibody (Clone BMA03, Immunotech, Marseille, France).

7. Cytokine measurement

For cytokine measurement, 10^5 T cells and 5×10^4 autologous, irradiated EBV-B cells were mixed in 200 µl of complete RPMI-FCS medium containing appropriate Ag concentration. After overnight incubation, supernatants were

collected and tested for the concentration of IL2, IFNγ and IL4. IL2 was quantified on a IL2-dependent CTLL line. IFNγ was measured by a sandwich ELISA as described (12). IL-4 was measured using the Duo-Set ELISA kit provided by Genzyme Diagnostics, Cambridge, MA. Eventually, cells were kept in culture for another 24 h and cellular proliferation was measured by thymidine uptake as described above.

8. Preferential expansion of PBMC-derived IL4-producing cells upon stimulation with different doses of Pen G

Patients BAM, CAS and ES participating to this study developed allergic reactions after treatment with β-lactam antibiotics and their clinical data are summarized in Table I. At the time of diagnosis they all presented different symptoms after treatment with β-lactams as well as positive LTT in vitro (Table I; ref. 2, 4 and data not shown). The Pen G specificity of their allergic reactions was assessed by RAST and skin tests which gave clear positive results for donors BAM and ES, but were negative for donor CAS. For all the three donors an immediate type of hypersensitivity was diagnosed, associated to delayed type reactions for donor BAM. Primary in vitro cultures were prepared stimulating purified PBMC with different doses of Pen G. The hapten was given in three fold dilutions starting from 3 mg/ml down to 0.1 μg/ml concentration, in two parallel cultures. After 5 days of incubation, the proliferative response was measured in one of the cultures by thymidine incorporation. As shown in Fig. 1, PBMC proliferated in response to Pen G in the range from 1 to 0.01 mg/ml, depending on the donor. The intensity of the response was dose-dependent, with maximal proliferation at 0.3 mg/ml of Pen G.

The second culture was further expanded in IL2-containing medium for 4 days. Cells were then washed and rested in the absence of IL2. After another 4 days, cells were harvested, restimulated with PMA and ionomycin, and analysed for intracellular IFNγ and IL4 expression by flow cytometry. TCR$\alpha\beta^+$ cells constituted up to 90 % of the cell populations and

they were homogeneously represented in the different cell lines (Fig. 1). As shown in the right panels of Fig. 1, upon in vitro stimulation with Pen G, mainly $IL4^+$ and $IL4^+IFN\gamma^+$ cells are expanded, while the growth of IFNγ-producing cells was rather unaffected. Comparing left and right panels of Fig. 1, the expansion of IL4-producing cells directly correlated with the intensity of the proliferative response. This indicating that, in our system, Pen G preferentially induced the recruitment of IL4-producing cells from peripheral blood lymphocytes and the intensity of their expansion depended on the dose of hapten used to induce the primary response in vitro. Interestingly, the preferential expansion of Pen G-specific, IL4-producing cells was associated to immediated type of hypersensitivity reactions in all the patients analyzed (Table I).

9. Effect of "hapten doses" on T cell clones

In order to evaluate the role of Pen G doses in inducing functional differentiation of established T lymphocytes, a panel of independent Pen G-specific T cell clones was prepared. PBMC from donors BAM and ES were subjected to two rounds of stimulation, in vitro, with Pen G at concentrations of 1, 0.3 and 0.1 mg/ml in three independent T cell lines. After expansion in IL2-containing medium, each line was independently cloned by limiting dilution. A total of 18 Pen G-specific T cell clones were obtained from donor BAM and 24 from donor ES. The hapten-specific T cell phenotype was induced stimulating each T cell clone with 3-fold dilution of Pen G, from 3 mg/ml to 0.03 mg/ml concentration, in the presence of irradiated autologous EBV-B cells. After 24 h incubation, supernatants were harvested and processed for cytokine measurement, while cells were kept in culture for another 24 h and T cell proliferation was measured by thymidine uptake. We then examined IL4 and IFNγ secretion induced in the presence of the highest and lowest Pen G dose able to trigger cellular proliferation, considering SI values ≥ 2.0 as positive response. Results are reported in Table II.

Clones from donor BAM showed strong proliferation in response to the highest dose of hapten (3 mg/ml), with SI values up to 122.7. For these clones the lowest dose of Pen G capable of triggering cellular proliferation was 0.03 mg/ml and, even at this low concentration, SI values of proliferation wer up to 86.5, indicating the high sensitivity of these reactivities. Phenotypically, BAM clones expressed a strong preference for type 0 or type 1 responses, and half of the clones were able to secrete both IL4 and IFNγ at high and low hapten doses. Interestingly, lower Pen G concentration induced higher level of IFNγ secretion in 14 of the 18 clones analyzed. This dose-dependent cytokine pattern resulted in a hapten-specific modulation of the T cell phenotype, and remarkably, 5 of the clones analyzed were able to shift from type 0 to type 1 responses.

Clones from donor ES resulted less sensitive to the hapten, being unable to proliferate to Pen G at concentrations < 0.3 mg/ml. For these the lowest Pen G doses capable of triggering cellular proliferation were ≥ 0.3 mg/ml with SI never exceeding 8.6. Clones ES mounted type 2 responses but could secrete IL4 only at the highest dose of Pen G tested (3 mg/ml).

Altogether, the hapten-specific T cell phenotypes were independent of the dose of Pen G used during in vitro induction of PBMC but depended on the donor analyzed. Thus, from donor BAM, who suffered of delayed as well as immediate type of hypersensitivity reactions, it was possible to isolate, after two cycles of restimulation with the specific hapten, IFNγ-producing T cell clones. In contrast, from donor ES, characterized by immediated type of hypersensitivity, mainly IL4-producing T cell clones could be isolated. However, the concentration of Pen G was indeed capable of modulating the T cell phenotypes. In fact, very high doses of Pen G were required to induce IL4 production by ES clones, while low doses of hapten induced higher level of IFNγ secretion, resulting in a shift of T cell phenotypes.

10. Pen G can modulate the T cell phenotype of fully differentiated hapten-specific T cell clones

To rule out if the antigen-driven phenotype modulation was a peculiar characteristic of the hapten under investigation, we further examined the effect of "antigen doses" on two other $CD4^+$ T cell clones.

Clone BAM25 was isolated 2 years ago in our laboratory and has been already described as Pen G-specific (7). clone 1ES4 derived from stimulation of peripheral blood cells of donor ES with the tetanus toxoid peptide sequence 616-630. The two clones were stimulated in vitro with different concentrations of either the specific Ag or the T cell mitogen PHA and cytokines released in the culture supernatant were measured after 24 h incubation.

As shown in Fig. 2, the cytokine patterns of clone BAM25 depended on the dose of Pen G used for stimulation. This clone was raised with a dose of Pen G of 1 mg/ml (7) and behaved, in this condition, as a Th2 clone, secreting IL4 but not IFNγ. However, the same clone simultaneously secreted IL4 and IFNγ in the presence of lower doses (< 0.3 mg/ml) of Pen G (Fig. 2A). In contrast, PHA stimulation induced a dose-dependent production of both IL4 and IFNγ (Fig. 2B), indicating the importance of the hapten-specific stimulation in defining Th0 or Th2 profiles. On the other hand, clone 1ES4 secreted IFNγ in response to both the specific antigen as well as the mitogen in a dose-dependent way (Fig. 2C-D).

Thus, the modulation of the T cell phenotype observed seems to be a peculiar characteristic of our hapten system. It appears feasible to drive the cytokine profile of long-term established Pen G-reactive T cell clones, altering the dose of hapten used for in vitro stimulation.

11. Antigen-independent IFNγ downmodulation by Pen G and V

The possibility to modulate the phenotype even of fully differentiated T cell clones could certainly introduce new strategies to manipulate immune responses. However, the data presented above apply exclusively to Pen G specificities. In order to find a system of more general application, the inventors also investigated the possibility to use other penicillin derivatives in addition to Pen G to modulate different antigen-specific immune reactions.

In a first set of experiments we studied the effect of the Pen V and Amp derivatives on the T cell phenotype induced by Pen G on two hapten-specific $CD4^+$ cell clones. Upon stimulation with 0.25 mg/ml of Pen G, clone BAM25 secreted IL2, IL4 and IFNγ (Fig. 3A-C), while clone ES5.13 produced IL2 and IL4 (Fig. 4A-B). None of them responded to neither Pen V nor Amp (Fig. 3A-C and Fig. 4A-B, respectively). In the assay, graded concentrations of Pen V and Amp were used together with 0.25 mg/ml of Pen G for stimulating T cell clones and the cytokines secreted in the culture supernatants were measured after 24 h incubation.

Results obtained from clone BAM25 are shown in Fig. 4D-F. Addition of Amp did not affect the Pen G-induced cytokine pattern, except for a slight reduction of IFNγ secretion at high concentration (Fig. 3D-F). In contrast, Pen V reduced the amount of IL2 of about 50 % (Fig. 3D) and completely abrogated IFNγ production at concentrations $\geq$ 0.1 mg/ml, without affecting IL4 secretion (Fig. 3E-F). The same type of assay, performed on clone ES5.13, revealed no interference of either Pen V or Amp on Pen G-induced IL2 secretion (Fig. 4C) and only a marginal reduction of IL4 production with high Pen V concentration (Fig. 4D).

Considering the structural similarities of the different penicillin molecules one might expect that the strong effect on IFNγ downregulation observed with Pen V could be due to altered interactions with the antigen-binding

sites of penicillin-specific TCR. In order to determine if this was the case, we performed the same assay described above, with the tetanus toxoid-specific T cell clone 1ES4. Upon stimulation with either the peptide sequence TT 616-630 at 1μg/ml and PHA at 0.3 μg/ml, this clone has a classical Th1 phenotype (Fig. 2). Surprisingly, even for these reactivities, the addition of Pen V at $\geq$ 0.1 mg/ml completely abrogated IFNγ secretion in response to either the specific antigen (TT peptide 616-630, Fig. 5B) and the mitogen (PHA, Fig. 5D), while IL2 production was unaffected (Fig. 5A,C). Again, addition of Amp had no effect (Fig. 5).

Thus, a complete inhibition of IFNγ secretion could be induced by addition of Pen V on two T cell clones of totally different specificities, i.e. Pen G (clone BAM25) or tetanus toxoid (clone 1ES4). Therefore, the effect observed is more likely independent of interaction with the TCR, but rather due to a direct interference of Pen V with IFNγ secretion pathways.

Downmodulation of the IFNγ production by PHA-stimulated T cells could be achieved also upon addition of Pen G, as shown in Fig.6. Similarly to what previously shown for Pen V, the IL-2 secrection was unaffected (Fig. 6, A and B). Amp, tested in the same experiment, showed again no effect on both IFNγ- and IL-2 secretion.

12. Implications

It has long been known that antigen dose can influence whether cell mediated or humoral responses are elicited and this largely depends on the development of $CD4^+$ T lymphocytes producing distinct sets of cytokines (8). In the mouse system, very high doses of antigenic peptides direct the differentiation of naive T cells into IL4-producing cells, while low levels of antigen promote the expansion of IFNγ-producing cells (9, 10). These findings suggested that antigen dose can alter the cytokine synthesis in naive $CD4^+$ T cells and even the induction and progression of drug-specific

adverse immune responses could be determined at the primary encounter with the hapten.

In the present examples the inventors focussed on the human allergic response to β-lactam antibiotics investigating the influence of hapten doses first, on the recruitment of drug-specific cells from PBMC of allergic donors, secondly, on the induction of T cell phenotype of established T cell clones.

All the donors participating in this study suffered from immediated type of hypersensitivity to β-lactams, and in particular to Pen G. Upon in vitro stimulation with different doses of Pen G we found a preferential recruitment of IL4-producing peripheral blood-derived T cells. This expansion was maximal for high-middle doses of hapten (around 1-0.1 mg/ml concentration) and directly correlated with the proliferative response of the cultures. This suggests that the drug-specific cells contributing to the adverse reactions are mainly of type 2 phenotype, as expected from the clinical characteristics of all the donors. Remarkably, this effect was particularly evident for donor CAS who developed an anaphylactic shock after treatment with β-lactams.

In those experiments the inventors were able to analyze only the contribution of memory T cells to penicillin-specific allergic responses. To rule out how these drug-specific reactions are induced at the very early encounter with the hapten and how hapten concentrations might influence T cell differentiation, it could be certainly interesting to investigate the effect of Pen G on human cord blood-derived naive T cells.

The role of Pen G doses in inducing functional differentiation of established T lymphocytes was analyzed on a panel of 42 independent T cell clones isolated from Pen G-specific after two cycles of restimulation with the hapten. It was found that the concentration of Pen G during in vitro induction does not affect the type of clones isolated, this depending only

on the donor studies. However, once T cell clones have been isolated and expanded, the dose of Pen G used for stimulation was, indeed, a critical factor determining their functional, hapten-specific phenotype. In fact, only high doses of Pen G (3 mg/ml) induced IL4 secretion and the type 2 clones isolated behaved as "inert" cells at low hapten concentration. On the other hand, low Pen G concentrations induced higher level of IFNγ secretion; as a consequence, 35% of clones isolated from donor BAM were able to shift their phenotype from type 0 to type 1 (Table II). Remarkably, the phenotype shift was very peculiar for penicillin-specific responses, since it was not observed with a tetanus toxoid-specific $CD4^+$ clone (i.e. clone 1ES4) and even not by PHA stimulation (Fig. 2). Moreover, the phenomena observed depended mainly on the modulation of IFNγ secretion as shown in detail for clone BAM25 which could secrete IFNγ only at Pen G concentration $\leq$ 0.3 mg/ml (Fig. 2).

For a correct interpretation of these data, one should consider how hapten-derived epitopes are formed and how the strength of their interaction with specific TCR is determined, in comparison to nominal peptidic Ag (13, 14). On a given APC, the density of epitopes formed by hapten-modification will depend, at least, on two factors: the number of modifiable sites and the concentration of reactive hapten molecules. At saturation, the density of epitopes will not increase with the concentration of the hapten. On the other hand, multiple modification might interfere with the strength of TCR ligation. We already found that a peptide sequence carrying multiple penicilloyl groups was a poor stimulator compared to a designer penicilloyl-peptide carrying only the preferential modified lysin residue, for the same clone (15). Taken together, these considerations suggest that for drug-specific immune responses there is no direct correlation between concentration of hapten molecules, epitope density and strength of TCR engagement.

Several recent studies focus on the identification of factors and signals capable of reversing Th responses (16, 17). In the inventor's system, the phenotype-modulation observed for Pen G-specific immune responses is, most probably, TCR-mediated.

In the last set of experiments presented, it was possible also to identify at least two penicillin derivatives capable of inducing a modulation of the T cell phenotype, with a TCR-independent mechanism. In fact, a dose-dependent inhibition of IFNγ production was observed when Pen V was added to manipulate either a Pen G-specific immune response (clone BAM25 in Fig. 3) as well as a tetanus toxoid-specific response or even a mitogen-induced phenotype (clone 1ES4 in Fig. 5). Similarly, also Pen G could downmodulate IFNγ production of mitogen-activated T cells (Fig. 6). In all these systems a downregulation of IFNγ secretion was observed, with no effect on IL4 production. The broad range of specificities which could be manipulated speaks in favour of TCR-independent mechanisms for Pen V-mediated IFNγ downmodulation. A similar effect has been very recently described for other compounds, such as pirydinil imidazole drugs. One of these compounds, SB203580, selectively induces IFNγ downmodulation in mouse Th1 cells through specific inhibition of p38 MAP kinases (18). It is tempting to speculate that similar targets could be modified also by Pen V.

Taken together, the above data indicate that penicillins can modulate the T cell phenotype of fully differentiated T cell clones in vitro, with TCR-dependent and TCR-independent mechanisms, thus representing potentially new tools for immune intervention. In particular, the possibility to selectively downmodulate IFNγ production via TCR-independent pathways offers a broad application for therapeutic intervention in autoimmune diseases and other pathological situations where a predominant Th1 profile is found.

Figure 1

Pen G-specific response of PBMC from donors allergic to β-lactams: effect of hapten doses on the recruitment of IL4-producing cells. Purified PBMC from donors BAM, CAS and ES were cultured, in vitro, with different doses of Pen G in two parallel cultures. Proliferative responses measured on the first culture are shown in the left panels; data are expressed in cpm x 10^3 and given as mean of triplicates, SD values are indicated. Cells in the second culture were further expanded, then rested in the absence of IL2 and finally harvested, restimulated with PMA and ionomycin, and analysed for IFNγ and IL4 productions by flow cytometry. Data are shown in the right panels and represented as percentage of IL4 (black bars), IFNγ (white bars) and IL4/IFNγ (grey bars) producing cells. For all the cultures the frequency of TCR$\alpha\beta^+$ cells, reported on the right, was determined by surface staining.

Figure 2

Pen G can modulate the phenotype of an established $CD4^+$ hapten-specific T cell clone. Clones BAM25 (A,B) and 1ES4 (C,D) were analysed for cytokine secretion in response to different concentrations of the specific antigen, Pen G (A) or TT616-630 peptide (C) respectively, and PHA (B,D). IFNγ and IL4 productions are shown in the left and right panels respectively. Data are expressed as pg/ml of secreted cytokines and given as mean of triplicates, SD values are indicated.

Figure 3

Effect of Pen V and Amp on the Pen G-specific phenotype of clone BAM25. IL2, IL4 and IFNγ productions in response to different concentration of Pen G (square), Pen V (circle) and Amp (triangle) are represented in A, B and C, respectively. In the experiment shown in the right panels, 10^5 T cells/w and 5 x 10^4 EBV-B cells/w were stimulated with 0.25 mg/ml of Pen G plus different amount of Pen V or Amp. IL2, IL4 and IFNγ productions, measured after 24 h incubation, are shown in D, E and F, respectively. Data are given as mean of triplicates and represented as % of cytokines secreted in the

presence of Pen G only. 100% values were: IL2 80.5 SI of CTLL cells, IL4 5788 pg/ml, IFNγ 6637 pg/ml.

Figure 4

Effect of Pen V and Amp on the Pen G-induced phenotype of clone ES5.13. IL2 and IL4 productions in response to different concentrations of Pen G (square), Pen V (circle) and Amp (triangle) are shown in A and B, respectively. The assay, shown in the right panels, was performed as described in Fig. 3. IL2 and IL4 productions, shown in C and D respectively are given as mean of triplicates and represented as % of cytokines secreted in the presence of Pen G only. 100% values were: IL2 40.3 SI, IL4 5414 pg/ml.

Figure 5

TCR-independent IFNγ downmodulation by Pen V. Clone 1ES4 was stimulated with the tetanus toxoid peptide sequence 616-630 at 1μg/ml (A,B) or PHA at 0.3 μg/ml (C,D) plus different doses of Pen V and Amp as described in Figure 3. IL2 and IL4 secretion after 24 h incubation are represented in A, C and B, D, respectively. 100 % values for TT 616-630 were: IL2 137.4 SI of CTLL cells, IFNγ 7497 pg/ml; for PHA: IL2 116 SI, IFNγ 7865 pg/ml.

Figure 6

Effects of penicillins on IFNγ production by fresh peripheral blood cells (PBMC).

Ficoll-purified PBMC ($1x10^5$) of a healthy donor were incubated with 1μg/ml phytohemeagglutinin (PHA) in the absence or presence of penicillin G (Pen G), penicillin V (Pen V) or Ampicillin (Amp) at the indicated concentrations. After 24 h at 37°C and 5% CO_2, supernatants were collected and IFNγ (A) or IL-2 (B) determined by ELISA. Maximal concentrations in the absence of antibiotics were 3.000 pg/ml for IFNγ and 600 pg/ml for IL-2. As shown, none of the antibiotics significantly affects IL-2 secretion while IFNγ

production is blocked almost completely by high concentrations of Pen G and Pen V, but not by Amp.

Table I Characteristics of the allergic individuals studied

Donor	Symptoms	IgE[a]	Skin Tests		Type of hyper-sensitivity
			Immediate[b]	Late[c]	
BAM	Urticaria Exanthema	+	-	+	I[e] and D[f]
CAS	Anaphylaxis	-	-	-	I
ES	Eodema	+	+	n.t.[d]	I

[a] Specific serum IgE as determined by RAST.

[b] Prick and i.d. tests for Pen G using either benzylpenicilloyl-polylysin or minor-determinant-mixture (MDM:benzylpenicilloyl/Pen G mixture).

[c] Patch test read after 48 h and 72 h.

[d] n.t. = not tested.

[e] I = immediated type hypersensitivity reaction.

[f] D = delayed type hypersensitivity reaction.

Table II Phenotypes of penicillin-specific T cell clones[a]

Clone[c]		PR (SI)[b]		Cytokines (ng/ml)				Type	
				IL4		IFNγ			
		max[d]	min[e]	max	min	max	min	max	min
BAM G1	B2	63.1	25.7	1.1	<[f]	7.6	8.8	TO	T1
	C12	87.7	56.2	4.1	2.2	6.5	8.6	TO	TO
	B10	46.5	33.0	6.6	4.6	6.4	7.9	TO	TO
	D6	41.2	4.8	1.7	<	5.2	5.9	TO	T1
	B12	81.8	6.7	5.5	4.1	6.6	8.2	TO	TO
	D7	68.3	48.6	1.3	0.5	8.4	8.6	TO	TO
	A10	101.6	18.8	4.2	<	7.6	<	TO	TO
	A6	95.4	81.2	5.0	4.2	8.6	10	TO	TO
	D10	122.7	86.5	7.5	6.9	7.2	8.7	TO	TO
BAM G0.3	D9	13.0	9.1	<	<	0.5	8.6	T1	T1
	D12	25.9	5.7	0.2	0.2	7	8.2	TO	TO
	B8	17.3	14.0	<	<	<	8.6	TO	T1
	C3	70.5	37.0	0.8	0.2	7.6	9.7	TO	TO
	A1	37.3	5.5	0.7	<	4.0	1.7	TO	T1
BAM G0.1	G11	75.4	9.5	<	<	7.1	7.9	T1	T1
	G4	56.4	12.2	7	<	3.4	5.4	TO	T1
	F3	77.1	7.1	10.8	<	<	<	T2	-
	F2	108.0	7.3	4.9	0.5	7.9	7.4	TO	TO
ES G1	A9	76.8	6.3	23.2	<	<	<	T2	-
	C6	16.4	7.6	<	<	<	<	T	-
	A8	19.0	3.0	1.0	<	<	<	T2	-
G0.3	F9	37.6	6.3	<	<	<	<	-	-
	F8	99.6	4.0	2.7	<	<	<	T2	-
	E12	90.7	5.3	1.7	<	<	<	T2	-
	H6	27.2	6.3	<	<	<	<	-	-
	H1	33.1	4.1	<	<	<	<	-	-
	E6	26.2	4.4	<	<	<	<	-	-
	E8	52.6	8.6	<	<	<	<	-	-
	H2	18.5	2.7	<	<	<	<	-	-
	H3	36.7	5.2	<	<	<	<	-	-
	F3	43.5	5.9	1.9	<	<	<	T2	-
	H10	72.4	3.2	<	<	<	<	-	-
G0.1	E6	24.3	2.0	<	<	<	<	-	-
	H3	91.6	6.7	2.7	<	<	<	T2	-
	H11	101.9	2.8	27.9	<	<	<	T2	-
	G2	105.1	6.3	<	<	<	<	-	-
	G4	141.0	5.0	5.3	<	<	<	T2	-
	H10	162.1	8.1	4.6	<	<	<	T2	-
	H8	32.0	3.2	1.1	<	<	<	T2	-
	E7	195.8	3.7	3.1	<	<	<	T2	-
	H6	50.6	4.1	2.7	<	<	<	T2	-
	H7	70.2	2.2	0.9	<	<	<	T2	-

[a] The in vitro test for cytokine production was set up in 96-well plates with 10^5 T cells, 5 x 10^4 irradiated autologous EBV-B and 3 fold dilutions of Pen

G from 3 mg/ml to 0.03 mg/ml. After 24 h supernatants were harvested for cytokine measurement, while cells were kept in culture for another 24 h and T cell proliferation was measured by thymidine inrorporation. The values reported refer to cytokine production in the presence of the highest (max) and lowest (min) hapten doses able to trigger cellular proliferation.

[b] PR: proliferative response; SI: stimulation index.

[c] The name of the clones indicate: the donor of origin, the dose of Pen G used for in vitro priming and the personal code.

[d] max: highest Pen G concentration tested; 3 mg/ml for all clones.

[e] min: lowest Pen G dose able to trigger cellular proliferation; 0.03 mg/ml for BAM clones; $\geq$ 0.3 for clones ES.

[f] <: not detectable.

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Claims

1. Use of at least one penicillin for the production of pharmaceutical for therapeutic use in autoimmune diseases or other pathological situations where IFNγ-mediated effects are involved in the progression of the disease.

2. Use according to claim 1, wherein the penicillin is PenG or PenV.

3. Use of a hapten-modified peptide for the production of a pharmaceutical for therapeutic use in autoimmune diseases or other pathological situations where IFNγ-mediated effects are involved in the progression of the disease
characterized in that it contains a backbone of amino acids wherein to at least one of these amino acids a penicillin antibiotic is bound.

4. Use of a hapten-modified peptide for the diagnostic determination of a predisposition for hypersensitivity reactions against penicillins or parts or derivatives thereof,
characterized in that it contains a backbone of amino acids wherein on at least one of these amino acids a penicillin antibiotic is bound.

5. Use according to claim 4, wherein PBMC cells are isolated from patients for whom a predisposition for hypersensitivity reactions is to be determined, the cells are stimulated in vitro by adition of the hapten-modified peptide and proliferation of antigen responsive T cells is measured.

6. Use according to claims 4 or 5, wherein IFNγ and/or IL4 expression are measured.

7. Use of a hapten-modified peptide for the desensitization of patients suffering from hypersensitivity reactions against penicillins or parts of derivatives thereof
characterized in that it contains a backbone of amino acids wherein on at least one of these amino acids a penicillin antibiotic is bound.

8. Use according to anyone of claims 3 to 7, wherein the penicillin antibiotic is penicillin G, penicillin V or ampicillin.

9. Use according to anyone of claims 3 to 8, wherein the amino acid backbone consists of 8 to 20 amino acids, preferably 10 to 18 amino acids.

10. Use according to anyone of claims 3 to 9, wherein the amino acid backbone contains at least one lysine onto which the antibiotic is bound.

11. Use according to anyone of claims 3 to 10, wherein the antibiotic is bound to amino acids at position 3, 5 or 8 starting from a tyrosine anchor.

FIG.1

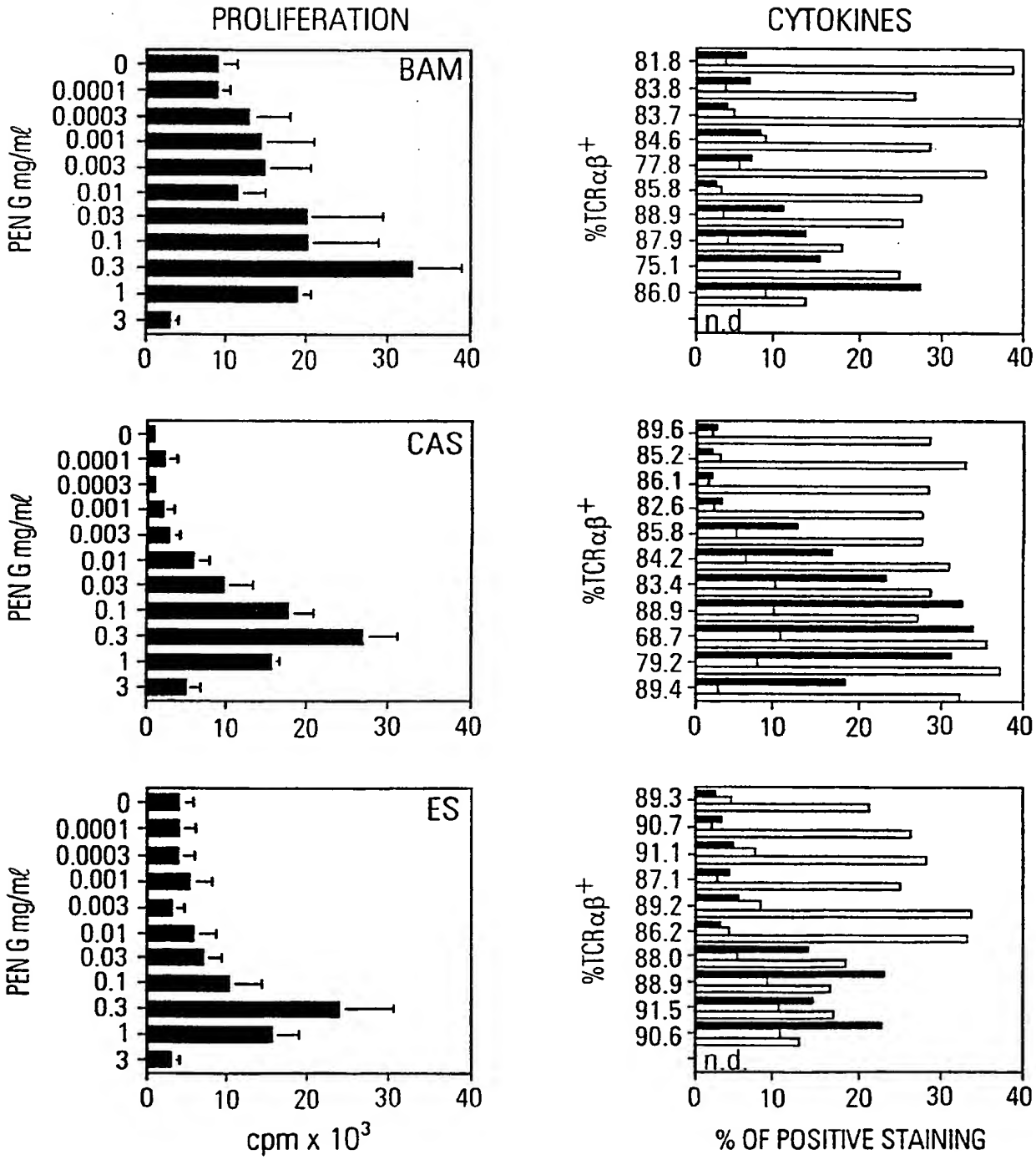
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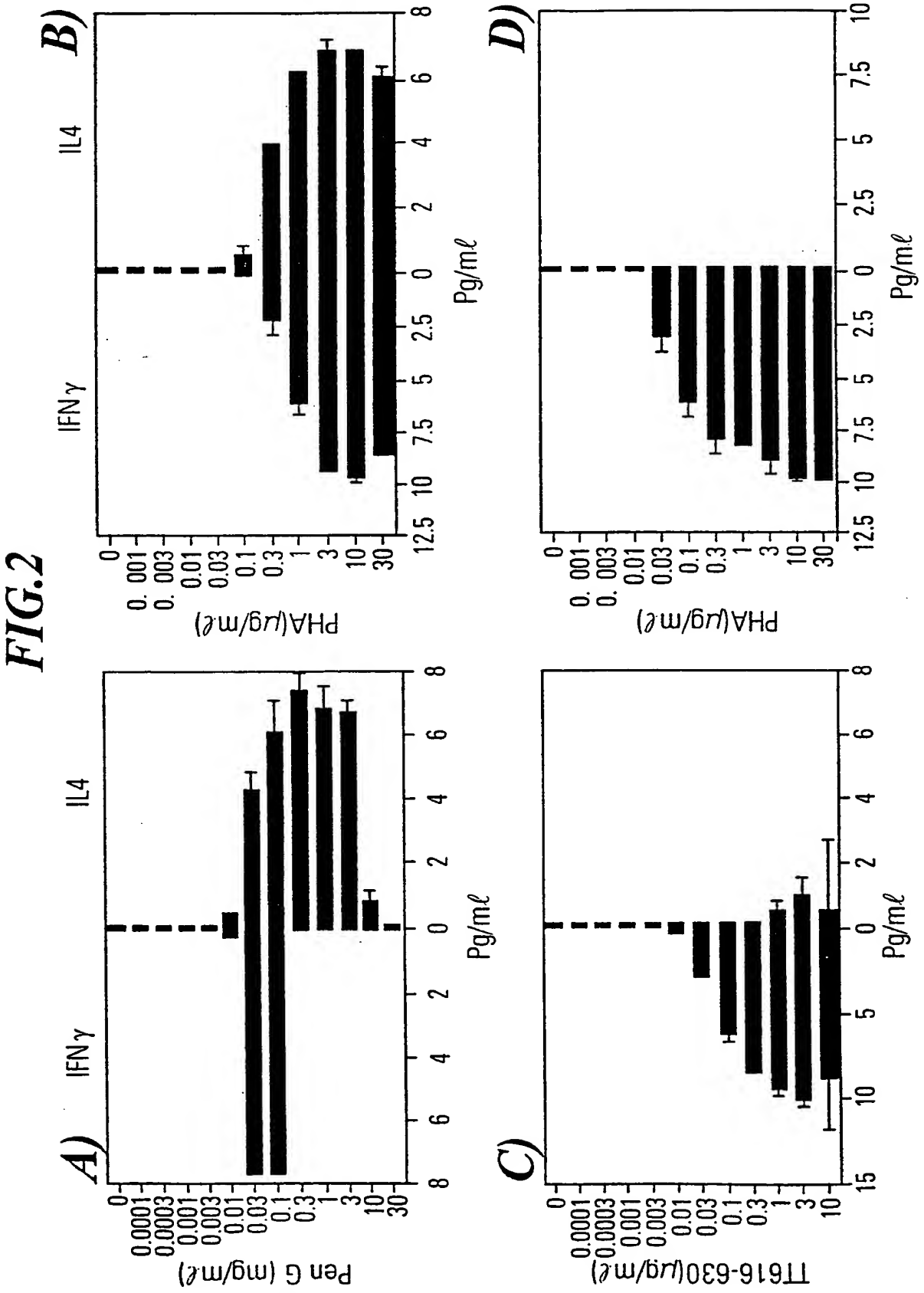
FIG.2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IL4
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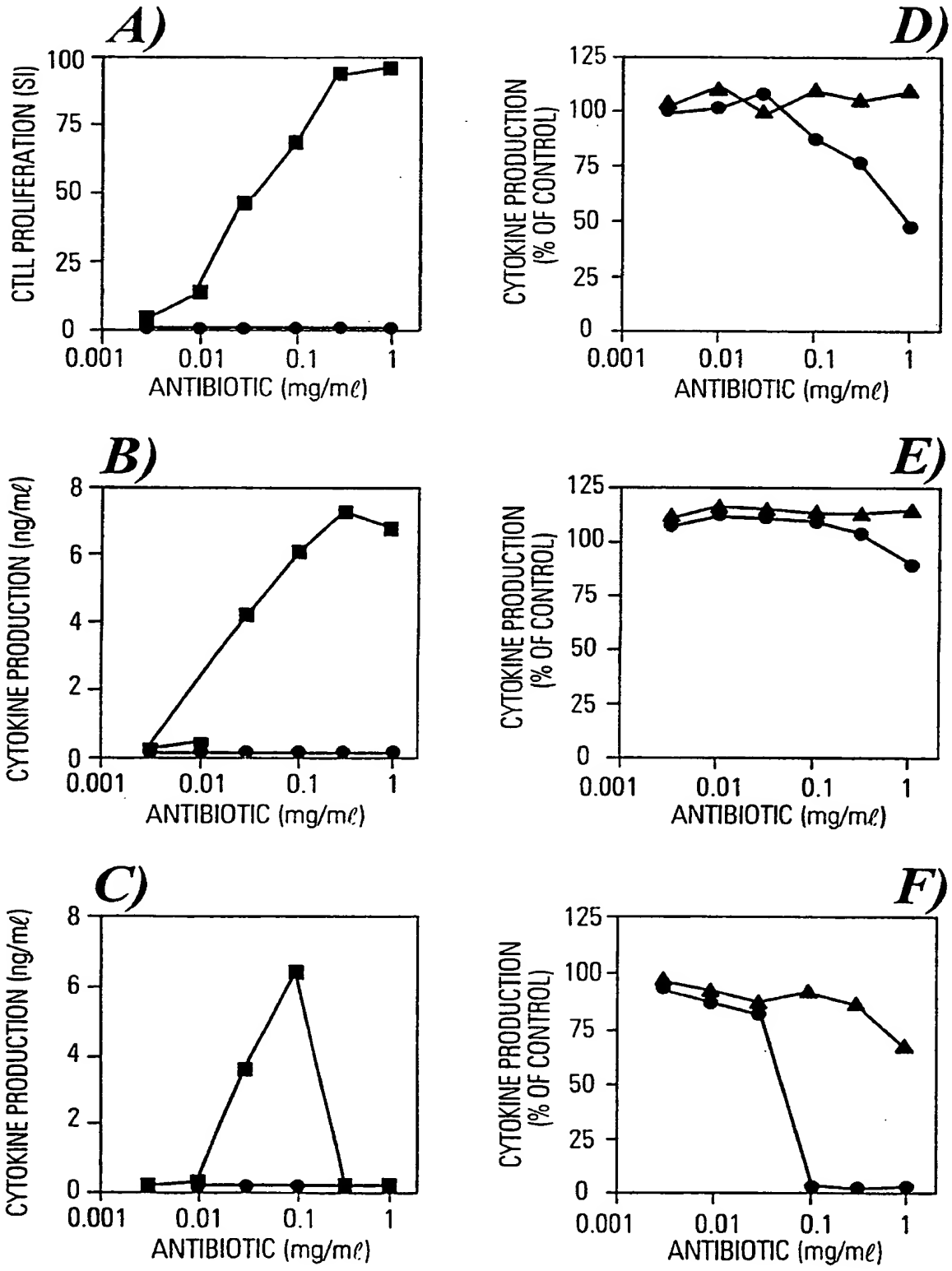
Pg/mℓ
B)
IFNγ
IL4
PHA(µg/mℓ)
Pg/mℓ
C)
TT616-630(µg/mℓ)
Pg/mℓ
D)
PHA(µg/mℓ)
Pg/mℓ

FIG.3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D)
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ANTIBIOTIC (mg/mℓ)
B)
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E)
CYTOKINE PRODUCTION (% OF CONTROL)
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ANTIBIOTIC (mg/mℓ)
C)
CYTOKINE PRODUCTION (ng/mℓ)
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ANTIBIOTIC (mg/mℓ)
F)
CYTOKINE PRODUCTION (% OF CONTROL)
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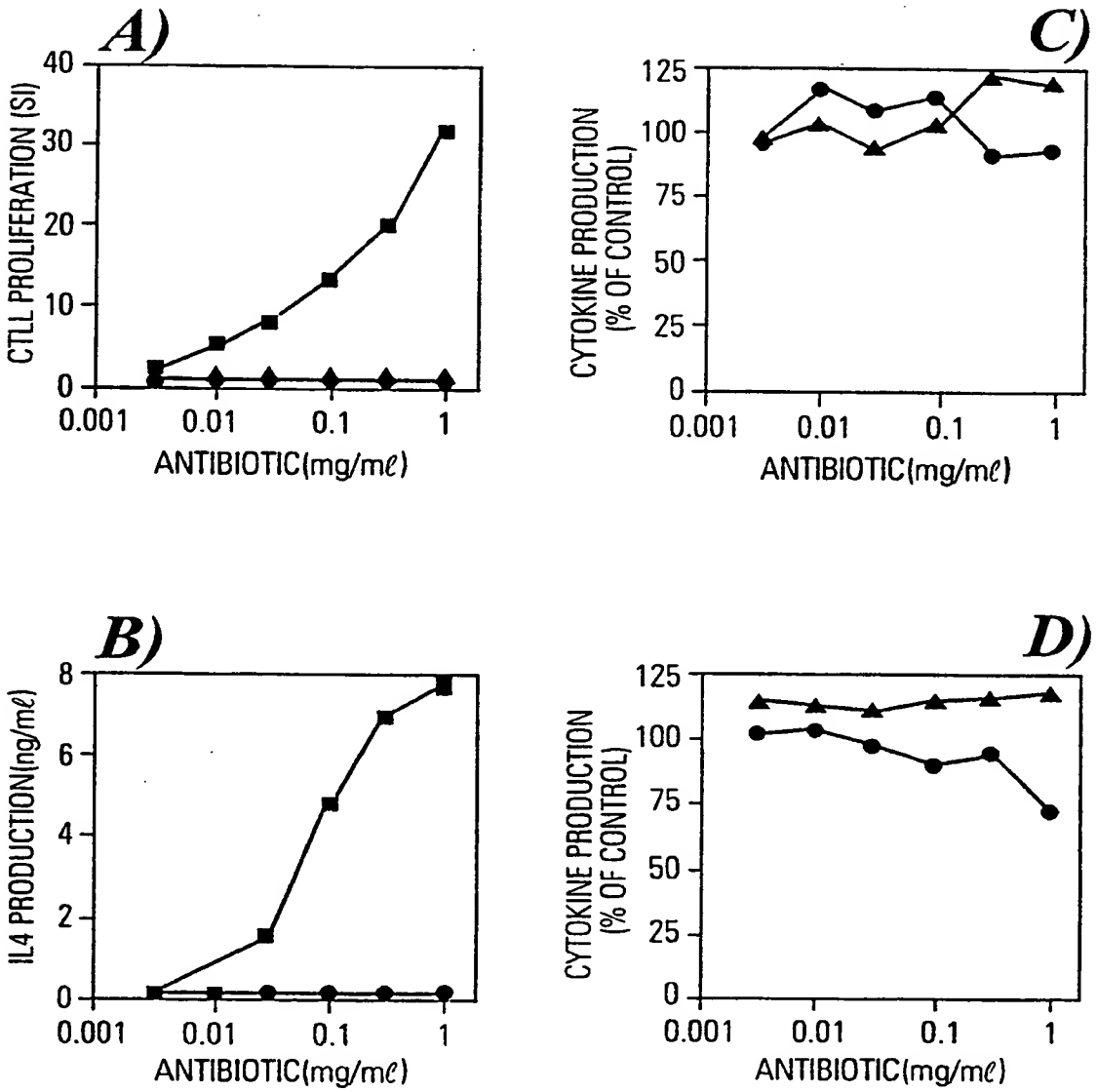
FIG.4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C)
CYTOKINE PRODUCTION (% OF CONTROL)
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ANTIBIOTIC(mg/mℓ)
B)
IL4 PRODUCTION(ng/mℓ)
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ANTIBIOTIC(mg/mℓ)
D)
CYTOKINE PRODUCTION (% OF CONTROL)
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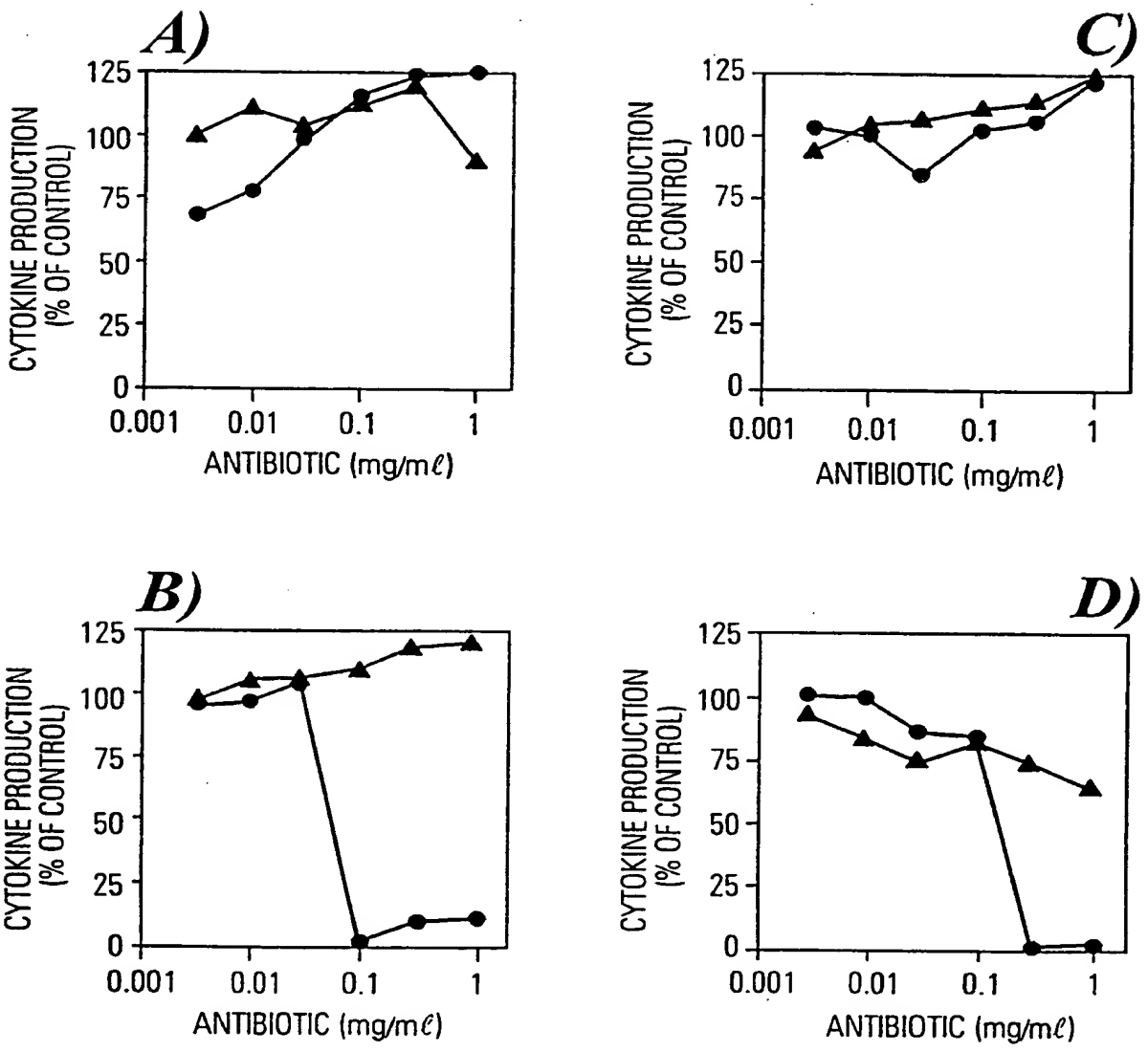
ANTIBIOTIC(mg/mℓ)

FIG.5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C)
B)
D)
CYTOKINE PRODUCTION (% OF CONTROL)
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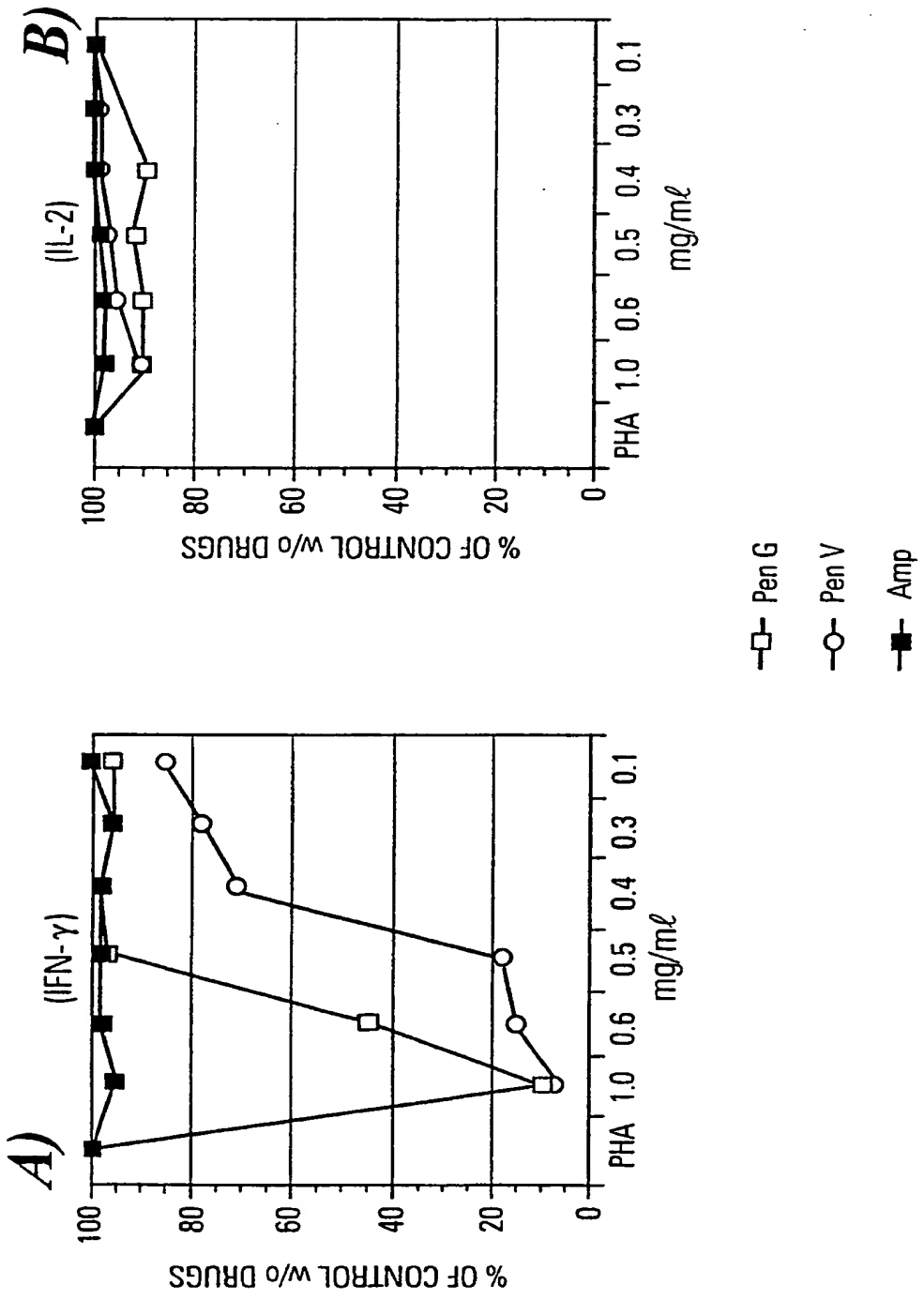
0.01
0.1
1
ANTIBIOTIC (mg/mℓ)

FIG.6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(IFN-γ)
B)
(IL-2)
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80
60
40
20
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PHA 1.0 0.6 0.5 0.4 0.3 0.1
mg/mℓ
Pen G
Pen V
Amp

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/EP 99/10378

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K7/08 C07K7/06 G01N33/50 A61K47/48 //G01N33/94

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PADOVAN ET AL.: "Penicilloyl peptides are recognized as T cell antigenic determinants in penicillin allergy" EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 27, June 1997 (1997-06), pages 1303-1307, XP002104706 cited in the application	1-3,8-11
Y	abstract page 1303, right-hand column -page 1304, left-hand column	4-6
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2000	12/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BRANDER ET AL.: "Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals" THE JOURNAL OF IMMUNOLOGY, vol. 155, 1995, pages 670-2678, XP002104707 cited in the application abstract page 2676, left-hand column, line 5 - line 7	4-6
A	GB 2 057 425 A (LEVINE B B) 1 April 1981 (1981-04-01) abstract; claims 12,17	1-11
A	FR 2 574 185 A (WECK ALAIN DE) 6 June 1986 (1986-06-06) abstract; claim 1; examples II,III	1-11
A	EP 0 309 299 A (CAMBRIDGE LIFE SCIENCES) 29 March 1989 (1989-03-29) abstract; claim 20	2,3,8
A	US 5 534 496 A (LEE VINCENT H ET AL) 9 July 1996 (1996-07-09) abstract; claims 1,3,7 column 3, line 21 - line 24	2,3,8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/10378

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

As far as claims 7-11 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

information on patent family members

Intern. Application No
PCT/EP 99/10378

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2057425 A	01-04-1981	US 4316882 A	23-02-1982
		DE 3000749 A	19-03-1981
		FR 2464258 A	06-03-1981
		FR 2464267 A	06-03-1981
FR 2574185 A	06-06-1986	NONE	
EP 0309299 A	29-03-1989	AU 2484488 A	18-04-1989
		WO 8903040 A	06-04-1989
		GB 2210372 A	07-06-1989
US 5534496 A	09-07-1996	NONE	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
19073P WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 10378	23/12/1999	23/12/1998

Applicant

MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER...et al.

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

national application No.
PCT/EP 99/10378

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

As far as claims 7-11 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

INTERNATIONAL SEARCH REPORT

[redacted]nal Application No
PCT/EP 99/10378

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K7/08 C07K7/06 G01N33/50 A61K47/48 //G01N33/94

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PADOVAN ET AL.: "Penicilloyl peptides are recognized as T cell antigenic determinants in penicillin allergy" EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 27, June 1997 (1997-06), pages 1303-1307, XP002104706 cited in the application	1-3,8-11
Y	abstract page 1303, right-hand column -page 1304, left-hand column	4-6
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2000	12/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ceder, O

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	BRANDER ET AL.: "Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals" THE JOURNAL OF IMMUNOLOGY, vol. 155, 1995, pages 670-2678, XP002104707 cited in the application abstract page 2676, left-hand column, line 5 - line 7	4-6
A	GB 2 057 425 A (LEVINE B B) 1 April 1981 (1981-04-01) abstract; claims 12,17	1-11
A	FR 2 574 185 A (WECK ALAIN DE) 6 June 1986 (1986-06-06) abstract; claim 1; examples II,III	1-11
A	EP 0 309 299 A (CAMBRIDGE LIFE SCIENCES) 29 March 1989 (1989-03-29) abstract; claim 20	2,3,8
A	US 5 534 496 A (LEE VINCENT H ET AL) 9 July 1996 (1996-07-09) abstract; claims 1,3,7 column 3, line 21 - line 24	2,3,8

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/10378

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2057425 A	01-04-1981	US 4316882 A	23-02-1982
		DE 3000749 A	19-03-1981
		FR 2464258 A	06-03-1981
		FR 2464267 A	06-03-1981
FR 2574185 A	06-06-1986	NONE	
EP 0309299 A	29-03-1989	AU 2484488 A	18-04-1989
		WO 8903040 A	06-04-1989
		GB 2210372 A	07-06-1989
US 5534496 A	09-07-1996	NONE	

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
19073P WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/10378	23/12/1999	23/12/1998
International Patent Classification (IPC) or national classification and IPC C07K7/08		
Applicant MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/06/2000	28.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fayos, C Telephone No. +49 89 2399 2180

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/10378

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-26	as originally filed	

Claims, No.:

1-6,8-11	as originally filed	
7	with telefax of	07/12/2000

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	-

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1- Reference is made to the following documents:

D1: PADOVAN ET AL.: 'Penicilloyl peptides are recognized as T cell antigenic determinants in penicillin allergy' EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 27, June 1997 (1997-06), pages 1303-1307, XP002104706 cited in the application

D2: BRANDER ET AL.: 'Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals' THE JOURNAL OF IMMUNOLOGY, vol. 155, 1995, pages 670-2678, XP002104707 cited in the application

NOVELTY - Art. 33 (1) and (2) PCT

2- **Claims 1-11 appear to be novel in the light of the prior art cited in the search report and for the following reasons:**

2.1- D1 discloses (p 1303 c 2 § 3 - p 1304 c 2 § 3 - Materials and Methods) hapten-modified peptides characterized in that they contain a backbone of 12 aminoacids wherein, on a lysine residue (at position 2, 4 and 7 starting from a tyrosine anchor), a penicillin (Pen G, Pen V or ampicillin) is bound and discloses the following experimental procedures:

- isolation of PBMC cells
- stimulation of cells in vitro
- measurement of antigen responsive Tcells and
- measurement of cytokine production (IL-4 and IFNγ)

D1 does hence describe penicilloyl peptides and the fact that they are recognised as Tcell antigenic determinants. D1 relates to the investigation of mechanisms which play a role in allergy development.

However, D1 differs from the present application in that the present application shows that such peptides can advantageously be used in vitro assays for the determination of a predisposition for hypersensitivity reaction against penicillins or parts thereof whereas D1 merely provides an insight into the manner in which allergenic haptens are recognized by human T cells involved in allergic reactions to drugs and suggests possible mechanisms leading to the onset of these adverse immune responses.

Hence, the claimed subject matter appears to be novel over D1.

2.2- D2 discloses (p 2671 c 1 § 3 - p 2672 c 1 - Materials and Methods) the coupling of penicillin G to BPO-HSA and the use of the obtained hapten-modified peptides for the diagnostic determination of a predisposition for hypersensitivity reactions against penicillins or parts or derivatives thereof comprising the steps of:

- isolation of PBMC cells
- stimulation of cells in vitro
- measurement of antigen responsive Tcells and
- measurement of cytokine production (IL-4 and IFNγ among others)

However, D2 differs from the present application in that unlike BPO-HSA, penicilloyl-peptides are molecularly defined reagents as far as the backbone structure is concerned and the position of the penicilloyl group is concerned, therefore allowing (i) the modification of the backbone sequence in order to achieve the highest binding capacity to donor-specific HLA molecules, (ii) the arbitrary choice of the site of penicilloyl modification along the backbone peptide according to the T cell reactivity to detect and (iii) that the degree of modification (number of penicilloyl groups) will depend on the modifiable lysine residues introduced into the sequence, and therefore is quantitatively defined.

Hence, the subject matter claimed appears to be novel over D2.

2.3- Finally, the use of a penicillin and of a hapten(penicillin)-modified peptide for the production of a pharmaceutical for therapeutic use in in autoimmune diseases or other pathological situations where IFNγ-mediated effects are involved in the progression of the disease and for desensitization of patients suffering from hypersensitivity reactions against penicillins or parts or derivatives thereof is also not explicitly disclosed in the prior art cited in the search report.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

3- The present application is based on the finding at least 2 penicillin derivatives are capable of inducing a modulation of the T cell phenotype, with a TCR-independent mechanism.

In fact, a dose-dependent inhibition of IFNγ production was observed when PenV was added to manipulate either a PenG-specific immune response as well as a tetanus toxoid-specific response or even a mitogen-induced phenotype. Similarly, also PenG could downmodulate IFNγ production of mitogen-activated T cells. In all these systems, a down regulation of IFNγ secretion was observed, with no effect on IL-4 production (see p 17 lines 6-15, and p 12-14 of the description of the present application).

3.1- The problem posed in the present application is therefore to provide pharmaceutical means for treating patients suffering from diseases which are caused by overreactions or malfunctions of the immune system (such as autoimmune diseases or other pathological situations where IFNγ-mediated effects are involved).

The solution proposed in the present application is the use of penicillin, alone or bound to a backbone of aminoacids.

For the treatment of diseases which are caused by overreactions or malfunctions of the immune system, penicillins have not yet been described and the present applications appears to disclose for the first time that penicillins can be used to down modulate de IFN-γ expression.

The closest prior art is represented by D1 which discloses some of the experimental procedures of the present application.

4- Claims 1-11 appear to be inventive for the following reasons:

4.1- D1 does not show/suggest that PenG and/or PenV can downmodulate IFNγ secretion and hence does not anticipate the subject matter claimed in the present application.

4.2- D2 makes clear that pen G specific T cell clones produce a heterogeneous cytokine pattern (see p 2675 c 2 and table 2 see also item 2.2- above) and hence does not explicitly suggest the subject matter of the present application.

4.3- Therefore, claims 1-11 can be considered as being inventive.

INDUSTRIAL APPLICABILITY - Art. 33 (1) and (4) PCT

5- Claims 1-11 appear to be industrially applicable.

Re Item VIII
Certain observations on the international application

6- There is an inconsistency between the subject matter claimed and p 2 lines 29-30 of the description of the present application (Art. 6 PCT).

New claim 7

7. Use of a hapten-modified peptide for the production of a pharmaceutical for the desensitization of patients suffering from hypersensitivity reactions against penicillins or parts of derivatives thereof
characterized in that it contains a backbone of amino acids wherein on at least one of these amino acids a penicillin antibiotic is bound.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

WEICKMANN, H.
WEICKMANN WEICKMANN HUBER LISKA
PRECHTEL BÖHM WEISS TIESMEYER
Kopernikusstrasse 9
D-81679 München
ALLEMAGNE

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	28.02.2001
Applicant's or agent's file reference 19073P WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/10378	23/12/1999	23/12/1998

Applicant
MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER...et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hundt, D Tel.+49 89 2399-8042

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
19073P WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/10378	23/12/1999	23/12/1998
International Patent Classification (IPC) or national classification and IPC C07K7/08		
Applicant MAX-PLANCK-GESELLSCHAFT ZUR FÖRDERUNG DER...et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 1 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/06/2000	28.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fayos, C Telephone No. +49 89 2399 2180

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/10378

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-26	as originally filed	

Claims, No.:

1-6,8-11	as originally filed	
7	with telefax of	07/12/2000

Drawings, sheets:

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	-

2. Citations and explanations
   see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1- Reference is made to the following documents:

D1: PADOVAN ET AL.: 'Penicilloyl peptides are recognized as T cell antigenic determinants in penicillin allergy' EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 27, June 1997 (1997-06), pages 1303-1307, XP002104706 cited in the application

D2: BRANDER ET AL.: 'Heterogeneous T cell responses to beta-lactam-modified self-structures are observed in penicillin-allergic individuals' THE JOURNAL OF IMMUNOLOGY, vol. 155, 1995, pages 670-2678, XP002104707 cited in the application

NOVELTY - Art. 33 (1) and (2) PCT

2- **Claims 1-11 appear to be novel in the light of the prior art cited in the search report and for the following reasons:**

2.1- D1 discloses (p 1303 c 2 § 3 - p 1304 c 2 § 3 - Materials and Methods) hapten-modified peptides characterized in that they contain a backbone of 12 aminoacids wherein, on a lysine residue (at position 2, 4 and 7 starting from a tyrosine anchor), a penicillin (Pen G, Pen V or ampicillin) is bound and discloses the following experimental procedures:

- isolation of PBMC cells
- stimulation of cells in vitro
- measurement of antigen responsive Tcells and
- measurement of cytokine production (IL-4 and IFNγ)

D1 does hence describe penicilloyl peptides and the fact that they are recognised as Tcell antigenic determinants. D1 relates to the investigation of mechanisms which play a role in allergy development.

However, D1 differs from the present application in that the present application shows that such peptides can advantageously be used in vitro assays for the determination of a predisposition for hypersensitivity reaction against penicillins or parts thereof whereas D1 merely provides an insight into the manner in which allergenic haptens are recognized by human T cells involved in allergic reactions to drugs and suggests possible mechanisms leading to the onset of these adverse immune responses.

Hence, the claimed subject matter appears to be novel over D1.

2.2- D2 discloses (p 2671 c 1 § 3 - p 2672 c 1 - Materials and Methods) the coupling of penicillin G to BPO-HSA and the use of the obtained hapten-modified peptides for the diagnostic determination of a predisposition for hypersensitivity reactions against penicillins or parts or derivatives thereof comprising the steps of:

- isolation of PBMC cells
- stimulation of cells in vitro
- measurement of antigen responsive Tcells and
- measurement of cytokine production (IL-4 and IFNγ among others)

However, D2 differs from the present application in that unlike BPO-HSA, penicilloyl-peptides are molecularly defined reagents as far as the backbone structure is concerned and the position of the penicilloyl group is concerned, therefore allowing (i) the modification of the backbone sequence in order to achieve the highest binding capacity to donor-specific HLA molecules, (ii) the arbitrary choice of the site of penicilloyl modification along the backbone peptide according to the T cell reactivity to detect and (iii) that the degree of modification (number of penicilloyl groups) will depend on the modifiable lysine residues introduced into the sequence, and therefore is quantitatively defined.

Hence, the subject matter claimed appears to be novel over D2.

2.3- Finally, the use of a penicillin and of a hapten(penicillin)-modified peptide for the production of a pharmaceutical for therapeutic use in in autoimmune diseases or other pathological situations where IFNγ-mediated effects are involved in the progression of the disease and for desensitization of patients suffering from hypersensitivity reactions against penicillins or parts or derivatives thereof is also not explicitly disclosed in the prior art cited in the search report.

INVENTIVE STEP - Art. 33 (1) and (3) PCT

3- The present application is based on the finding at least 2 penicillin derivatives are capable of inducing a modulation of the T cell phenotype, with a TCR-independent mechanism.

In fact, a dose-dependent inhibition of IFNγ production was observed when PenV was added to manipulate either a PenG-specific immune response as well as a tetanus toxoid-specific response or even a mitogen-induced phenotype. Similarly, also PenG could downmodulate IFNγ production of mitogen-activated T cells. In all these systems, a down regulation of IFNγ secretion was observed, with no effect on IL-4 production (see p 17 lines 6-15, and p 12-14 of the description of the present application).

3.1- The problem posed in the present application is therefore to provide pharmaceutical means for treating patients suffering from diseases which are caused by overreactions or malfunctions of the immune system (such as autoimmune diseases or other pathological situations where IFNγ-mediated effects are involved).

The solution proposed in the present application is the use of penicillin, alone or bound to a backbone of aminoacids.

For the treatment of diseases which are caused by overreactions or malfunctions of the immune system, penicillins have not yet been described and the present applications appears to disclose for the first time that penicillins can be used to down modulate de IFN-γ expression.

The closest prior art is represented by D1 which discloses some of the experimental procedures of the present application.

4- Claims 1-11 appear to be inventive for the following reasons:

4.1- D1 does not show/suggest that PenG and/or PenV can downmodulate IFNγ secretion and hence does not anticipate the subject matter claimed in the present application.

4.2- D2 makes clear that pen G specific T cell clones produce a heterogeneous cytokine pattern (see p 2675 c 2 and table 2 see also item 2.2- above) and hence does not explicitly suggest the subject matter of the present application.

4.3- Therefore, claims 1-11 can be considered as being inventive.

INDUSTRIAL APPLICABILITY - Art. 33 (1) and (4) PCT

5- Claims 1-11 appear to be industrially applicable.

Re Item VIII

Certain observations on the international application

6- There is an inconsistency between the subject matter claimed and p 2 lines 29-30 of the description of the present application (Art. 6 PCT).

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

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in its capacity as elected Office

Date of mailing (day/month/year) 28 July 2000 (28.07.00)	
International application No. PCT/EP99/10378	**Applicant's or agent's file reference** 19073P WO
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Applicant PADOVAN, Elisabetta et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

06 June 2000 (06.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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